

**128-Plat****Observing Real-Time Force-Dependent Conformational Changes of Single Integrin on Living Cells and Their Regulation of Ligand Dissociation**Wei Chen<sup>1</sup>, Jizhong Lou<sup>2</sup>, Evan A. Evans<sup>3</sup>, Cheng Zhu<sup>1</sup>.<sup>1</sup>Georgia Institute of Technology, Atlanta, GA, USA,<sup>2</sup>Institute of Biophysics, Chinese Academy of Sciences, Beijing, China,<sup>3</sup>Boston University, Boston, MA, USA.

Integrins are adhesion molecules that connect a cell to its environment and transduce signals bidirectionally across the membrane. Their different functional states correspond to distinct conformations. Using a biomembrane force probe, we observed real-time reversible switches between bent and extended conformations of single cell surface integrin  $\alpha$ L $\beta$ 2 by measuring its nanometer-scale headpiece displacements, (un)bending frequencies and molecular stiffness changes. We determined stabilities of these conformations, their dynamic equilibrium, speeds and rates of conformational changes, and impacts of divalent cations and tensile forces. We quantified how initial conformations and their subsequent changes of  $\alpha$ L $\beta$ 2 regulate the force-dependent kinetics of dissociation from intercellular adhesion molecule 1. Our findings provide new insights into how integrins function as nanomachines to precisely control cell adhesion and signaling.

**129-Plat****Conformational Change of HIV Nef upon Insertion into Lipid Membranes Resolved by Neutron Reflectivity**Michael S. Kent<sup>1</sup>, Bulent Akgun<sup>2</sup>, Hirsh Nanda<sup>2</sup>, Joseph E. Curtis<sup>2</sup>, Sushil Satija<sup>2</sup>, Xiaomeng Shi<sup>3</sup>, John R. Engen<sup>3</sup>.<sup>1</sup>Sandia National Labs, Albuquerque, NM, USA, <sup>2</sup>National Institute of Standards and Technology, Gaithersburg, MD, USA,<sup>3</sup>Northeastern University, Boston, MA, USA.

Nef is one of several HIV-1 accessory proteins and directly contributes to AIDS progression. Nef has no catalytic activity but instead realizes its functions by interacting with cellular proteins. Nef is myristoylated on the N-terminus, associates with membranes, and undergoes a transition from a solution conformation to a membrane-associated conformation. It has been hypothesized that conformational rearrangement enables membrane-associated Nef to interact with cellular proteins. Despite its obvious disease importance, there is little or no direct information about the conformation of membrane-bound Nef. In this work we used neutron reflection to reveal details of the conformation of membrane-bound Nef. The conformation of myristoylated Nef was studied upon binding to Langmuir monolayers of negatively-charged lipids. By adjusting the surface pressure, the extent of insertion of the myristate group could be controlled. At sufficiently high surface pressure such that the myristate group did not insert, adsorbed Nef was in a condensed state with the core domain directly against the lipid headgroups. At lower surface pressure such that the myristate group inserted into the membrane, adsorbed Nef was found to be in an extended state with the core domain displaced  $\sim 70$  Å from the lipid headgroups. Thus, insertion of the myristate group triggers a conformational transition to an open configuration. This has important ramifications for the ability of Nef to interact with host proteins.

**130-Plat****Molecular Mechanisms Behind Mitochondrial Fission**

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Mitochondria undergo a remarkable sequence of fission and fusion events during its lifecycle yet the molecular basis of these events is just emerging. Fis1 appears essential to mitochondrial fission by directly or indirectly recruiting Drp1, a dynamin-related mechanoenzyme to sites of scission. A genetic screen in yeast identified non-functional alleles of Fis1 alleles but the root of this misfunction is not known. We have taken biased and unbiased approaches to define Fis1 mechanism, which have converged on a new model. Specifically, we determined that these non-functional alleles unexpectedly form elevated amounts of dimer in vitro compared to wild type, suggesting that enhanced dimerization of Fis1 interferes with mitochondrial fission. We rationally designed a single point mutation that disrupts in vitro dimerization of Fis1. Surprisingly, either obligate monomers or dimers are impaired in fission, indicating that either the failure to dimerize or excessive dimerization interfere with Fis1 function. To dissect this further, we have developed a novel method to rapidly identify essential Fis1 residues. We randomly generated a library of Fis1 variants and simultaneously screened for disruption of Fis1-mediated yeast two-hybrid interactions with each binding partner of the  $>3000$  colonies screened,  $\sim 9\%$  selectively disrupted interactions with one of the three protein partners. To test the functional consequence of this analysis, we parsed each hit sequence into its corresponding single point mutations and tested viability in

a growth assay that directly reports on mitochondrial fission of 97 Fis1 mutants tested to date, 40 resulted in nonfunctional fission indicating these residues are essential for mitochondrial fission. An analysis of these mutations supports a new model for the assembly of the mitochondrial fission machinery. We anticipate that this method will be useful in defining critical residues in other macromolecular machines and “signaling hub” proteins with multiple binding partners.

**Platform: Membrane Structure I****131-Plat****Chemical Imaging of the Lipid and Cholesterol Distribution in the Plasma Membranes of Intact Cells**Mary L. Kraft<sup>1</sup>, Jessica F. Frisz<sup>1</sup>, Kaiyan Lou<sup>1</sup>, Haley A. Klitzing<sup>1</sup>, William P. Hanafin<sup>1</sup>, Peter K. Weber<sup>2</sup>, Joshua Zimmerberg<sup>3</sup>.<sup>1</sup>University of Illinois, Urbana, IL, USA, <sup>2</sup>Lawrence Livermore National Laboratory, Livermore, CA, USA, <sup>3</sup>National Institutes of Health, Bethesda, MD, USA.

Domains enriched with cholesterol and sphingolipids, often referred to as lipid rafts, are believed to be present in the plasma membranes of eukaryotic cells and mediate important cellular functions, including cell signaling and virus budding. Changes in the abundances of cholesterol and sphingolipids influence many cellular processes. Yet, the distributions of cholesterol and sphingolipids within the plasma membrane have not been established. The inability to directly visualize lipids and cholesterol without the use of labels that may induce clustering renders characterizing the precise distribution of cholesterol and sphingolipids within the plasma membrane especially challenging. To address this problem, we have previously combined a chemically specific and spatially well-resolved imaging technique, high-resolution secondary ion mass spectrometry (SIMS), with metabolic labeling in order to image the distributions of sphingolipids within the plasma membranes of intact cells with at least 100 nm lateral resolution. We now report the use of this approach to image the sphingolipid and cholesterol distributions in parallel on the surface of intact fibroblast cells. We also quantitatively assess whether the cholesterol and sphingolipids are heterogeneously distributed and co-localized within the membrane. The results of these studies and their implications on models of membrane organization will be discussed.

**132-Plat****Protein Micropatterning in the Plasma Membrane Allows for Kd Determination in Living Cells and Superresolution Analysis of Lipid Rafts**Stefan Sunzenauer<sup>1</sup>, Mario Brameshuber<sup>2</sup>, Julian Weghuber<sup>3</sup>, Gerhard J. Schuetz<sup>2</sup>.<sup>1</sup>Institute for Biophysics, Linz, Austria, <sup>2</sup>Institute of Applied Physics, Vienna University of Technology, Vienna, Austria, <sup>3</sup>Austria University of Applied Sciences, School of Engineering and Environmental Sciences, Wels, Austria.

In 2008 we introduced an assay for quantitative analysis of the interaction between a fluorescently marked protein (prey) and a membrane protein (bait) using microstructured surfaces, produced by  $\mu$ -contact printing, covered with ligands (antibodies) targeted against the bait. The proof-of-concept was demonstrated for the interaction between CD4, a major co-receptor in T cell signaling, and Lck, a protein tyrosine kinase essential for early T cell signalling (Schwarzenbacher, et al., Nat Methods, 2008).

Since then we worked on technical advances of the assay which are presented here. A whole binding curve of secondary antibodies binding to immobilized mouse antibodies was recorded, demonstrating that our assay allows for determination of the dissociation constant between a bait and a prey protein in living cells. Furthermore we show that our method is a powerful tool to study membrane micro domains or lipid rafts: Via patterning of GPI-anchored GFPs the structure of cells membrane was some kind of sorted and the behaviour of various proteins in these regions have been analyzed by super resolution microscopy.

**133-Plat****Creating and Modulating Membrane Microdomains in Pore-Spanning Bilayers**Alexander Orth<sup>1</sup>, Winfried Roemer<sup>2</sup>, Claudia Steinem<sup>1</sup>.<sup>1</sup>University of Goettingen, Goettingen, Germany, <sup>2</sup>University of Freiburg, Freiburg, Germany.

The architecture of the plasma membrane is not only determined by the lipid and protein composition, but is also influenced by its attachment to the underlying cytoskeleton. We show that microscopic phase separation of “raft-like” lipid mixtures in pore-spanning bilayers is strongly determined by the underlying highly ordered porous substrate. In detail, pore-spanning